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Summary

Echinococcosis is a re-emerging zoonotic disease in Kyrgyzstan. In 2012, an echinococcosis control scheme was started that included dosing owned dogs in the Alay Valley, Kyrgyzstan with praziquantel. Control programmes require large investments of money and resources; as such it is important to evaluate how well these are meeting their targets. However, problems associated with echinococcosis control schemes include remoteness and semi-nomadic customs of affected communities, and lack of resources. These same problems apply to control scheme evaluations, and quick and easy assessment tools are highly desirable. Lot quality assurance sampling was used to assess the impact of approximately two years of echinococcosis control in the Alay valley. A pre-intervention coproELISA prevalence was established, and a 75% threshold for dosing compliance was set based on previous studies. Ten communities were visited in 2013 and 2014, with 18-21 dogs sampled per community, and questionnaires administered to dog owners. After 21 months of control efforts, 8/10 communities showed evidence of reaching the 75% praziquantel dosing target, although only 3/10 showed evidence of a reduction in coproELISA prevalence. This is understandable, since years of sustained control are required to effectively control echinococcosis, and efforts in the Alay valley should be continued.

Keywords: *Echinoccocus*, control programme, Lot Quality Assurance Sampling (LQAS), Kyrgyzstan

Key findings

- Prior to control efforts, canine echinococcosis coproELISA prevalence was estimated at 20.1%
- Praziquantel dosing targets of 75% of owned dogs were met in 8/10 communities after ~2 years of control
- CoproELISA prevalence did not decrease in all communities, indicating the need for continued control
- Lot quality assurance sampling is a useful tool to evaluate the impact of echinococcosis control efforts

Introduction

Echinococcosis is a neglected zoonotic disease (WHO 2010) caused by infection with the larval stage of cestode tapeworms in the genus *Echinococcus* (Eckert et al. 2004). Human cystic and alveolar echinococcosis are caused by *E. granulosus* sensu lato (Alvares Rojas et al. 2014) and *E. multilocularis*, respectively (Eckert and Deplazes 2004), and humans are usually infected by eggs released in the faeces of an infected carnivore host, often domestic dogs (WHO/OIE 2001). Both diseases are characterized by the formation of cysts, usually in the liver or lungs (WHO/OIE 2001), and may be fatal if untreated (Fujikura 1991, Moro et al. 2009). Echinococcosis affects mainly pastoral communities worldwide, although the burden of the disease varies greatly in different locations (WHO/OIE 2001). Echinococcosis is relatively common in Central Asia (Torgerson et al. 2006, Torgerson 2013, Usubalieva et al. 2013), and is a public health concern in Kyrgyzstan (Torgerson et al. 2006). There are concerns the disease is re-emerging, and human cases of cystic and alveolar echinococcosis have increased greatly since Kyrgyzstan's independence from the Soviet Union in 1991 (Torgerson et al. 2006, Usubalieva et al. 2013, Raimkylov et al. 2015).

In 2011, the Kyrgyz Ministry for Agriculture and the World Bank considered echinococcosis to be of sufficient concern to implement an intervention programme which included providing anthelmintics for dogs (World Bank 2010). Dosing of domestic dogs with praziquantel (PZQ)

began in the summer of 2012, with an aim to dose all owned dogs four times a year (WHO 2011). When implementing control programmes, it is important to evaluate how well these are meeting their targets (Schantz et al. 1995, Schantz 1997). However, as echinococcosis often affects rural and remote communities (Craig et al. 2007), the same challenges associated with implementing the control scheme will affect the evaluation of the control scheme itself. Relatively quick and easy evaluation tools are therefore beneficial to assess the impact of echinococcosis control schemes (see also van Kesteren et al. 2015).

Coproantigen ELISAs have proved a useful diagnostic approach for canine echinococcosis (Allan et al. 2006, Craig et al. 2015). However, testing all dogs is difficult and therefore a sample of dogs is generally taken. In remote areas such as the Alay Valley, where communities and households may be scattered, it is difficult to attain large sample sizes for owned dogs not only because of logistical constraints, but also because many dogs are frequently free-roaming and people (and their dogs) may be semi-nomadic (van Kesteren et al. 2013). Lot quality assurance sampling (LQAS, Dodge et al. 1929), provides a statistically robust method of interpreting data despite requiring a relatively small sample size. The LQAS methodology has been adapted and simplified for application in field studies (Valadez et al. 2002), and has been applied to studies related to healthcare (see Robertson et al. 2006) and canine echinococcosis (van Kesteren et al., 2015). In order to evaluate the echinococcosis intervention programme, ten communities in the Alay Valley were visited in April 2013 and April 2014 and LQAS methodology was applied to assess praziquantel dosing compliance, and canine echinococcosis coproELISA prevalence.

Methods

Scheme for control of echinococcosis

The Kyrgyz Ministry of Agriculture, with financial support from the World Bank, proposed an echinococcosis control programme (World Bank 2010). The proposal aimed to improve the livestock sector in Kyrgyzstan, specifically aiming to increase productivity and reduce zoonotic diseases (World Bank 2010). Funding was allocated to several programmes, including the development of a national disease control action plan, establishment of an animal disease surveillance system, implementation of a national public information campaign, and implementation of a comprehensive nationwide vaccination and testing program for eight diseases of livestock and/or dogs (foot and mouth disease, anthrax, rabies, brucellosis, sheep pox, peste des petits ruminants, echinococcosis, and tuberculosis). One proposed project was the provision of anthelminthics to domestic dogs in an attempt to control echinococcosis (World Bank 2010).

Praziquantel tablets were provided to local community veterinarians at regional centres (for Alay Valley communities this was either Daroot Korgon or Gulcha), with community veterinarians instructed to go around to the households in their communities once in each season (winter, spring, summer and autumn) and either dose household dogs themselves or leave tablets with dog owners and instruct them to dose their dogs if dogs were not present at the time (T. Sultanov, Taldu Suu veterinarian, pers. comm.). In addition, veterinarians provided dog passports to monitor praziquantel dosing. The programme in the Alay Valley was considered a pilot project, and between 2013 and 2014, an estimated 7,610 dogs were registered with dog passports and dosed four times per year (unpublished data provided by the Kyrgyz Ministry of Agriculture and Land Reclamation and the Kyrgyz State Inspectorate for Veterinary and Phytosanitary Safety). In 2015, the dosing campaign was expanded to other parts of Kyrgyzstan by the Kyrgyz Ministry of Agriculture, as well as being continued in the Alay Valley. Parallel to the dosing campaign, dog

culling campaigns are implemented in Alay Valley communities. These are not specifically aimed at reducing echinococcosis but at controlling dog numbers. Prior to culls, which occur somewhat randomly during the year, and vary per village, community members are advised to tether or lock up their dogs for a specified period; untethered dogs are considered unwanted and culled (A. Gaitanbekov, Sary Mogul community veterinarian, pers. comm.).

Communities

Ten communities in the Alay Valley were selected as part of this study. All were situated along the major road (A327) that runs through the valley from west (the border with Tajikistan) to east (the border with Xinjiang, China). The communities sampled were (from west to east): Kyzyl Eshme (39.57°, 72.27°), Kabyk (39.59°, 72.39°), Achyk Suu (39.47°, 72.50°), Jaylima (39.62°, 72.59°), Kashka Suu (39.64°, 72.67°), Kara Kavak (39.66°, 72.72°), Sary Mogul (39.68°, 72.89°), Taldu Suu (39.70°, 72.98°), Archa Bulak (39.69°, 73.08°) and Sary Tash (39.73°, 73.25°) – see Figure 1. All communities were small villages with up to ~400 households, and populations of between a few hundred to at most ~3,000 people (see also van Kesteren et al. 2013, Mastin et al. 2015).

Establishing a pre-intervention coproELISA prevalence

Four communities (Taldu Suu, Sary Mogul, Kara Kavak and Kashka Suu) were visited in May 2012, prior to the start of the World Bank intervention programme (Mastin et al. 2015). All available households (i.e. those where occupants were at home at the time we visited) in Taldu Suu, Sary Mogul and Kara Kavak were visited, and all dogs present were sampled. If the occupants of a house were not home, we selected a neighbouring house and inquired about the presence or absence of dogs in unavailable households to be able to accurately assess the dog population. Due

to time constraints it was not possible to census all dogs In Kashka Suu. Instead, random locations within the community were selected and the six nearest available households were registered, with enquiries made about dog ownership of unavailable households at neighbouring households to be able to accurately assess the dog population. This process was continued until approximately 50 dogs had been registered in total. Based on estimation of total household numbers from satellite imagery, this process resulted in the registration of approximately 25% of all households in the villageThe number of dog faecal samples collected and analysed from each community was as follows: Kara Kavak=35, Kashka Suu=42, Sary Mogul=155, Taldu Suu=86 (Mastin et al. 2015).

Lot Quality Assurance Sampling: faecal sample and questionnaire data collection

A Lot Quality Assurance Sampling (LQAS) framework was adopted to evaluate the levels of canine echinococcosis in April 2013. A minimum of 19 dogs were sampled in Achyk Suu, Archa Bulak, Kabyk, Kyzyl Eshme, Jaylima and Sary Tash (a sample size of 19 is the smallest sample size that minimizes the risk of type A and B errors, see Valadez et al. 2002). To select dogs, a GPS coordinate for the centre of each community was determined using Google Earth images (based on imagery collected by the 'SPOT5' satellite in 2010). This location was taken as a starting point. Upon arriving at this point, the second hand on a watch was used to determine a random direction in which to walk, with a straight line then followed towards the edge of the community. Along this route, alternate households visited and if dogs were present they were sampled and questionnaires were administered to their owners. If a dead end or the end of the community was reached, the second hand of the watch was again used to determine at random a new walking direction and the same approach was used, until a minimum of 19 dogs had been sampled, with additional dogs sampled if time allowed (however one sample collected from Achyk Suu in 2013 was lost in transport).

In the remaining four communities (Taldu Suu, Sary Mogul, Kara Kavak and Kashka Suu), more extensive sampling was undertaken as part of another study in which all household sampled in 2012 prior to the dosing campaign were sampled again in spring and autumn 2013 and 2014 to collect more detailed information on *Echinococcus* spp. in these communities with an aim to create a mathematical model of transmission (Mastin 2015, and Mastin et al., in prep). For these communities, maps of visited households were used to recreate the LQAS sampling approach – again, by selecting a theoretical start point in the centre of the community, choosing a random direction (using a watch) and selecting 19 sampled households in that direction from the 'start point', and including any dogs in these households. The number of samples analysed per community was as follows (shown as 2013;2014): Kyzyl Eshme=19;19, Kabyk=19;19, Achyk Suu=18;19, Jaylima=19;21, Kara Kavak=21;19, Kashka Suu=19;19, Sary Mogul=19;19, Taldu Suu=19;19, Archa Bulak=19;19 and Sary Tash=19;19.

Dog owners were asked about the age and sex of their dogs, and when their dog was last dosed with PZQ. We expected that the start of the dosing campaign, as well as the start of an international research study on echinococcosis in the area, would increase awareness about echinococcosis in the local communities, as both veterinarians and researchers visiting local households often explained their work to dog owners. Also, the dosing campaign coincided with the appearance of public health notices on Kyrgyz television about echinococcosis (A. Gaitanbekov and T. Sultanov, local veterinarians, pers. comm). Therefore, in 2014, dog owners were also asked if they had heard of echinococcosis, and if they knew what caused the disease. Questionnaires were administered in Kyrgyz by a native speaker (Bermet Mytynova). Faecal samples were collected from around the

177 dog owner's homes and subsamples were stored in 0.3% PBS Tween (Fisher Scientific,
178 Loughborough, UK) with 10% formalin (sourced locally). Faecal samples were shipped at room
179 temperature to the University of Salford, UK.
180
181 The LQAS method was also used to determine whether the dosing programme was effectively
182 reaching people in each community. Although praziquantel dosing schemes may aim to reach all
183 owned dogs, it is unrealistic to assume a 100% compliance rate, with rates of <60% to >80%
184 previously reported from Kenya and China (see Torgerson 2003). The World Bank aimed to dose
185 dogs four times a year, and mathematical simulation models have shown that with dosing every 3-
186 4 months, a compliance rate of 75% can be effective in reducing transmission of echinococcosis
187 (Torgerson 2003, Torgerson et al. 2003a). For this reason we set our criterion at 75% of dogs dosed
188 in the four months prior to our visit. Because dog owners could often not remember the exact day
189 of dosing, only the month was noted and all reported dosings in January, February, March and
190 April were included as being within four months prior to our visit (samples were collected between
191 6 and 20 April 2013 and 5 and 12 April 2014). Where the latest dosing was not known, it was
192 assumed the dog had not been dosed in the previous four months.
193
194 **Choosing LQAS decision numbers**
195 Although simplified field manuals including decision numbers are available for LQAS sampling
196 (Valadez et al. 2002), it is possible to calculate decision numbers more accurately if the population
197 size and exact prevalence are known. This can be done using the hypergeometric distribution and
198 applying the following formula (from Lemeshow et al. 1991):

199 $$P(d \leq d^*) = \sum_{d=0}^{d^*} \frac{\binom{NP_0}{d}\binom{N(1-P_0)}{n-d}}{\binom{N}{n}}$$

200 Where N is the total dog population size in a community, P_0 is the prevalence threshold, n is the
201 number of dogs sampled, and d* is the decision number-1. The decision number must be an integer
202 and should be the lowest possible integer at which P is greater than or equals 10%. If d* or fewer
203 positive samples are obtained (i.e. if d is not reached), this is interpreted as some evidence that the
204 true prevalence is lower than P_0. For example, in Taldu Suu, a census of the dog population
205 revealed there were 98 dogs in the community (N=98), the dosing target was set at 75% (P_0=0.75),
206 and the number of dogs sampled was 19. By adjusting d in the equation above, the probability of
207 sampling at least d dosed dogs can be estimated, given that the true proportion of dosing was at
208 least 75%. For n=11:

209 $$\sum_{d=0}^{d^*} \frac{\binom{98 * 0.75}{11}\binom{98(1-0.75)}{19-11}}{\binom{98}{19}} = 0.06$$

210 For n=12:

211 $$\sum_{d=0}^{d^*} \frac{\binom{98 * 0.75}{12}\binom{98(1-0.75)}{19-12}}{\binom{98}{19}} = 0.16$$

212 As such the decision number for which P exceeds or equals 10% was calculated as 12, meaning
213 that any sampling round which found fewer than 12 dosed dogs would provide some evidence that
214 the true proportion of dosed dogs was lower than 75%. R code for calculation of the decision
215 number based upon the hypergeometric distribution is provided in the Supplementary Information.
216

In order to calculate N, data collected in May 2012 from Sary Mogul, Taldu Suu and Kara Kavak was used, and extrapolated from the randomly sampled houses in Taldu Suu (for details see Mastin et al. 2015). The size of the four villages was estimated using the 'measure distance' tool in Google Earth, to select the area that contained most of the houses. The number of dogs (from census data) was then divided by the area of the villages to give an estimate of the dog density (average of 1.56 dogs/100m^2, SE=0.30). The sizes of the other six communities were then estimated using Google Earth and the dog population estimated using the average dog density of 1.56 dogs/ha from the four main study villages (Table 1). It is worth noting that dog population numbers in the Alay Valley do fluctuate due to a bi-annual dog culling campaign. The data from May 2012 was collected just prior to dog culling and as such the dog population numbers shown in Table 1 are estimates extrapolated from populations that had not recently been culled. Because of this, and the irregular nature of the dog culling, we may have overestimated the dog populations present in some communities, which could affect decision numbers, particularly for communities with small dog populations. However sensitivity analysis found that even if dog populations were overestimated by a third, LQAS results for dosing compliance and coproELISA prevalence would not change, so uncertainty of exact population sizes is not considered further.

P_0 was set at the pre-intervention prevalence as calculated from the samples collected from Sary Mogul, Taldu Suu, Kashka Suu and Kara Kavak in 2012, and n was determined as the number of dogs sampled in each community in each year (18, 19 or 21). For praziquantel dosing, P_0 was set at 75% (Torgerson 2003, Torgerson and Heath 2003a).

CoproELISA

240 Details of the coproELISA are given in Mastin et al. (2015). Briefly, after decontaminating at -
241 80°C for ≥4 days (WHO/OIE 2001), faecal samples were extracted by homogenizing, shaking,
242 and centrifuging at 2500r.p.m (1125*g*) for 5 minutes, and collecting the supernatant. Faecal
243 samples were analysed for *Echinococcus* spp. coproantigen using a genus-specific sandwich
244 ELISA (see Allan et al. 1992, Craig et al. 1995, van Kesteren et al. 2015). Supernatants of two
245 known positive samples (one natural infection, one sample spiked with *E. granulosus* adult worm
246 extract) were used as positive controls throughout. Three known coproELISA negatives from a
247 very low endemic area (Falkland Islands) were also included as negative controls.
248
249 The 'pre-intervention' faecal samples collected in May 2012 and the post intervention samples
250 collected (LQAS) in April 2013 and April 2014 were analysed in two batches. Aliquots of all
251 reagents were pooled to a sufficient volume to test all the faecal samples in each lot and mixed, to
252 ensure minimum variation in coproELISA test conditions.
253
254 Cut-offs for OD values were determined for the coproELISA using ROC curve panels (Gardner et
255 al. 2006) of faecal samples of known infection status. These panels included arecoline purge
256 samples from dogs in the Alay Valley (van Kesteren et al. 2013) and samples from necropsied
257 dogs in communities in Hobukesar County, Xinjiang China (van Kesteren et al. 2015). The aim
258 was to compare the pre-intervention data to the data collected in April 2013 and 2014, and as such
259 cut-offs were chosen to give similar sensitivities (Se) and specificities (Sp) between the two
260 batches of samples, rather than choosing cut-offs that necessarily maximized Se/Sp. For the pre-
261 intervention samples (May 2012) a cut-off was chosen that gave a diagnostic sensitivity of 90%
262 and a specificity of 86%. For the post-intervention 'LQAS' samples (April 2013 and April 2014)

a cut-off was chosen that gave a diagnostic sensitivity of 89% and a specificity of 88% (Note that for this reason the pre-intervention coproELISA prevalence described here is different from that described in Mastin et al. 2015, which used a cut-off to maximize Se and Sp).

Results

Pre-intervention coproELISA prevalence

The dog faecal samples collected in May 2012 (n=318) gave an overall average coproELISA prevalence of 20.1%, with a within-village range from 16.3% in Taldu Suu to 22.9% in Kara Kavak. Village differences were ignored for the purposes of the current study, and the P_0 for coproELISA prevalence was therefore set at 20.1%

Dog demographics and praziquantel dosing in April 2013 and April 2014

A total of 191 dogs were sampled in April 2013. The majority of these (157 or 82.2%) were male, with 28 females (14.7%). For six dogs (3.1%) the sex was not recorded. Most dogs were younger than five years (131, or 69.3%, see Fig. 2, although the age of ten dogs was not recorded, and for 6 dogs neither age nor sex was recorded.

A total of 192 dogs were sampled in April 2014. The majority of these (156 or 81.3%) were male, with 35 females (18.2%). The sex of one dog (0.5%) was not recorded. Most dogs were younger than five years (156 or 81.3%, Fig. 3), and for 5 dogs the age/sex was not recorded.

In 2013, the majority of dog owners reported dosing their dog at some point in the seven months before sampling (141, or 73.8%, Fig. 4), with one person reportedly dosing their dog 11 months before sampling (0.5%). However 39 dog owners (20.42%) reported never dosing their dogs, and

a further 10 owners (5.2%) did not know when their dog had last been dosed, if ever (Fig. 4). In 2014, 152 dog owners (79.2%, Fig. 4) reported dosing their dog in the seven months before sampling, with four dogs (2.1%) being dosed between 7 and 8 months prior to sampling. In 2014, 23 dog owners (12.0%) reported never dosing their dogs and for a further 13 dogs (6.8%), the latest dosing was not known (Fig. 4).

Local knowledge of echinococcosis

In 2014, dog owners were asked whether or not they had heard of human echinococcosis, and what they thought caused human echinococcosis (open question). A total of 149 dog owners were asked these questions (some owners had multiple dogs, and some owners did not answer these questions). For the cause of echinococcosis, answers were classified as either 'correct', 'incorrect' or 'partially correct'. 'Correct' answers included: dog faeces, foxes, wolves, and contact with dogs. If owners correctly identified dogs and dog faeces as possible sources of infection but also listed incorrect sources such as sheep or mice (which are potential sources of canine echinococcosis, but not human infection), these were classed as 'partially correct'. If owners said they didn't know what caused echinococcosis, or gave wrong responses, for example 'livers' (which would be correct for canine echinococcosis but not human echinococcosis) then the answer was classed as 'incorrect'. Out of the 149 respondents, 126 (84.6%) had heard of echinococcosis, and 93 of these (78.3%) correctly identified causes of echinococcosis, with a further 13 respondents (10.3%) giving partially correct responses. 23 dog owners (15.4%) had not heard of echinococcosis and could not correctly identify its causes, but of the respondents who had heard of echinococcosis, 20 could also not correctly identify its causes. As such a total of 43 dog owners (28.9%) could not correctly identify causes of echinococcosis.

Using the LQAS method to evaluate PZQ dosing

Although the majority of dogs were dosed in the four months prior to sampling in 2013 (109, or 56.5%), there were marked differences between villages. None of 19 dogs were dosed in the previous four months in Sary Mogul in 2013, compared to 16 out of 19 dogs dosed in Jaylima in 2013 (Table 2). Six out of ten communities (Archa Bulak, Kara Kavak, Kashka Suu, Kyzyl Eshme, Sary Mogul, Sary Tash) did not meet the LQAS decision number for praziquantel dosing, suggesting that the praziquantel dosing scheme failed to reach at least 75% of owned dogs in these communities in 2013 (see Table 2).

In 2014, the overall proportion of dogs dosed no more than four months prior to sampling was higher than in 2013 (128, or 66.7%). Dosing compliance rates also seemed to have improved, with only two communities (Kashka Suu and Kyzyl Eshme) failing to meet the decision number (see Table 2). This suggests that the praziquantel dosing scheme was reaching more owned dogs in 2014 than in 2013.

Using LQAS to evaluate the impact of two years of intervention on coproELISA prevalence

The LQAS methodology described above was also used to evaluate whether the coproELISA prevalence had decreased following the start of the intervention programme. P_0 was set at 20.13% based on the pre-intervention sampling, and we aimed to identify villages that had achieved a reduction in their coproELISA prevalence.

In 2013, five communities in the Alay valley (Archa Bulak, Kara Kavak, Kashka Suu, Sary Mogul and Sary Tash) did not meet the decision number set according to LQAS requirements (Table 3). In 2014, three communities (Archa Bulak, Jaylima, and Sary Tash) did not meet the LQAS

330 decision number (Table 3). These results provide some evidence that the canine coproELISA
331 prevalence in these communities was lower than the pre-intervention value of 20.13%.
332
333 **Discussion**
334 Echinococcosis is a neglected zoonotic disease that can be fatal in humans (WHO/OIE 2001) and
335 can also have a large economic impact on rural communities due to the detrimental effects on
336 livestock productivity (Benner et al. 2010). Echinococcosis is re-emerging in Kyrgyzstan
337 (Torgerson et al. 2003b, Raimkylov et al. 2015) and was specifically mentioned as one focus of a
338 livestock disease control programme in the country (World Bank 2010). However, echinococcosis
339 is very difficult to control or eliminate (WHO/OIE 2001) especially in continental regions that are
340 relatively remote and where people are nomadic or semi-nomadic (Schantz et al. 2003, Craig et al.
341 2006). In these cases frequent praziquantel dosing of domestic dogs (standard recommended
342 dosing every six weeks) may not be practically feasible (Gemmell et al. 1986, Lembo et al. 2013),
343 and surveillance of the effectiveness of the scheme in the field is made even more challenging.
344
345 The implementation of control programmes for echinococcosis is costly in terms of both financial
346 and human resources, and as a result, control programmes have frequently not had the long term
347 success hoped for (Craig and Larrieu 2006). As such, it is important to evaluate the real impact of
348 control programmes, rather than focussing on easily-available metrics such as the amount of
349 money spent, or the number of praziquantel tablets distributed. Effective evaluation of control
350 programmes requires data to be collected from the communities in question, including reliable pre-
351 intervention data, and data collection should continue at suitable intervals during the control
352 programme itself. The data collected will depend on the questions being asked, but of particular
353 value are infection-centred measures such as the prevalence of canine infection (or the copro-

prevalence, as a proxy), or the prevalence of human echinococcosis. However, the challenges of implementing control programmes will also apply to the evaluations of control programmes. As such, relatively quick and easy tools to evaluate echinococcosis control programmes are highly desirable.

In order to evaluate the impact of the intervention programme in the Alay Valley, a pre-intervention coproELISA prevalence was established (van Kesteren et al. 2013, Mastin et al. 2015). To assess the impact of the control programme, ten communities were visited in April 2013 and April 2014 (~9 and 21 months after the start of the dosing scheme). From these, we aimed to assess praziquantel dosing compliance and coproELISA prevalence, with the praziquantel dosing threshold set at 75% of dogs dosed in the previous 3-4 months (Torgerson 2003). In 2013, four of the ten communities reached the decision number associated with this dosing target, and in 2014 this number had increased to eight out of ten. Although the LQAS methodology does not allow us to state that the target was reached for these communities, the number of communities for which there was evidence of the target not being met was lower in 2014 than 2013, which is suggestive that the dosing scheme was reaching at least 75% of owned dogs in most communities sampled. Furthermore, in 2014 a majority of dog owners (84.6%) had heard of human echinococcosis and could describe its causes (78.3%).

In 2013, there was evidence that the copro-prevalence was lower than the pre-intervention estimate of 20.13% in five out of ten communities sampled. However, in 2014, this had decreased to three out of the ten communities sampled, despite the higher number of communities reaching the threshold for reported praziquantel dosing. Although LQAS methodology, by virtue of the small

sample sizes collected, does not lend itself well to individual-level interpretation, it was reported that over half of the 33 dogs found to be coproELISA positive in 2014 were reported to have been dosed within the previous four months. This may reflect information biases from owners regarding the timing of dosing, errors in dosing (for example, tablets not swallowed or incorrect dosages administered), or reinfection. Although praziquantel is highly effective in treating canine echinococcosis, it provides no protection against reinfection, and if dogs continue to have access to offal and/or small mammals, they may become re-infected with *E. granulosus*, *E. canadensis* or *E. multilocularis*: all three of which are known to be transmitted in dogs in the Alay valley (van Kesteren et al. 2013). Deworming dogs using praziquantel is considered to eventually reduce the infection pressure to dogs through decreasing the infection pressure to livestock and small mammals, although this takes time due to the longevity of cysts in livestock (e.g. Torgerson and Heath 2003a). Similarly, although the lifespan of voles and other small mammals is much shorter than that of sheep (Bobek 1969, Devevey et al. 2009), it will take 1-2 years for infected small mammals to die off (Moss et al. 2013). Furthermore, *E. multilocularis* transmission is expected to be less responsive to dog dosing campaigns due to its sylvatic lifecycle (Eckert and Deplazes 2004). Therefore, even if dogs were correctly dosed, they may still be subject to high reinfection pressures, which may explain the poor correlation between reported praziquantel dosing and coproELISA prevalence.

When using LQAS, it is important to be aware of the limitations of this methodology. LQAS methodology remains statistically robust in the presence of small sample sizes by operating on the group level rather than the individual level, and by classifying groups (in the current study, villages) in a dichotomous fashion. As a result, conclusions can only be made at the level of the

village, and individual-level associations within these villages cannot be assessed. This latter issue means that although possible reasons for a lack of association between praziquantel dosing and coproantigen positivity at the individual dog level can be postulated (see above), further studies would be required to evaluate this more fully. Another important consideration in interpreting the results presented here is that of limitations in the diagnostic test itself. It has been well reported that the coproantigen ELISA functions best in the presence of higher worm burdens (Allan and Craig 2006). Control schemes using anthelminthics may affect the degree of overdispersion in a community since treatment of high burden individuals (which contribute most to overdispersion) bring the mean worm burden closer to the threshold for detection using the coproantigen ELISA, resulting in greater instability in the prevalence estimates obtained when using a single cut-off for 'positivity'. It should also be noted that as pre-intervention coproELISA prevalences were estimated from the four communities (Sary Mogul, Taldu Suu, Kara Kavak and Kashka Suu) sampled prior to the dosing campaign, we are not able to draw detailed conclusions about individual communities, which would require more extensive data collection (e.g. Mastin 2015, Mastin et al. 2015).

Surveillance of echinococcosis in domestic dogs allows for a practical evaluation of a control programme, with the benefit that dogs can be sampled and tested for *Echinococcus* spp. non-invasively through coproELISA analysis of faecal samples collected from the ground (e.g. Pierangeli et al. 2010). In addition, the application of novel sampling methodology like LQAS can reduce some of the laboriousness associated with evaluating control programmes, and provide a relatively quick and easy tool to test if control programmes are meeting their targets. Here we found evidence that a minority of villages failed to reach reasonable levels of praziquantel dosing

by 2014, suggesting that the echinococcosis control programme was reaching the other communities. Although analysis of the canine infection data did not show evidence of a gradual decrease in coproELISA prevalence over time, longer timescales are required to evaluate these changes. Effective control of echinococcosis takes years if not decades, and a sustained effort will be required to reduce infection pressures and effectively control cystic echinococcosis in the Alay Valley, and the co-endemicity with alveolar echinococcosis in the Alay valley (Usubalieva et al. 2013) also makes control more challenging. Fortunately control efforts in the Alay Valley by the Kyrgyz Ministry of Agriculture are ongoing, with an estimated ~6,000 and ~4,000 dogs treated in 2015 and 2016 respectively, with an estimated total of 24,162 and 15,501 praziquantel tablets provided to dogs (unpublished data provided by the Kyrgyz Ministry of Agriculture and Land Reclamation and the Kyrgyz State Inspectorate for Veterinary and Phytosanitary Safety). Following the initial project in the Alay Valley, the control programme has been expanded to other parts of Kyrgyzstan. The LQAS methodology described here would provide a relatively low-cost method of evaluating canine infection status over the coming years, given that the control scheme is maintained.

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Table1: Estimated dog populations in the 10 communities sampled.
** indicates known dog number from census*
*** indicates extrapolated dog number from sample*

Village name	**Estimated area (ha)**	**Estimated dog number**	**Estimated dog density (dogs per ha)**
Taldu Suu	37	98*	2.66
Sary Mogul	121	157*	1.30
Kara Kavak	31	35*	1.12
Kashka Suu	105	120**	1.14
Archa Bulak	16	25	1.57
Sary Tash	56	90	1.61
Kabyk	29	50	1.71
Kyzyl Eshme	68	105	1.56
Achyk Suu	61	95	1.56
Jaylima	17	30	1.74

Table 2: Dogs dosed in the four months prior to sampling in each of the ten communities in April 2013 and April 2014. Communities in bold type did not meet the LQAS requirements, meaning that for these communities there was evidence that fewer than 75% of households had recently dosed their dogs with praziquantel

2013				2014			
Community	PZQ in prev. 4 ms	No PZQ in prev. 4 ms	Decision #	Community	PZQ in prev. 4 ms	No PZQ in prev. 4 ms	Decision #
Achyk Suu	13	5	11	Achyk Suu	16	3	12
Archa Bulak	**11**	**8**	**13**	Archa Bulak	17	2	13
Jaylima	16	3	13	Jaylima	19	2	14
Kabyk	15	4	12	Kabyk	14	5	12
Kara Kavak	**10**	**11**	**14**	Kara Kavak	16	3	12
Kashka Suu	**10**	**9**	**12**	**Kashka Suu**	**5**	**14**	**12**
Kyzyl Eshme	**12**	**7**	**12**	**Kyzyl Eshme**	**4**	**15**	**12**
Sary Mogul	**0**	**19**	**12**	Sary Mogul	13	6	12
Sary Tash	**7**	**12**	**12**	Sary Tash	17	2	12
Taldu Suu	15	4	12	Taldu Suu	16	3	12

Table 3: CoproELISA positive and negative faecal samples in the ten communities sampled in April 2013 and April 2014. Communities in bold type met the LQAS requirements, meaning that for these communities there was no evidence of a decrease in coproantigen prevalence from the baseline of 20.1%

2013				2014			
Community	CoproELISA +ve	CoproELISA -ve	Decision #	Community	CoproELISA +ve	CoproELISA -ve	Decision #
Achyk Suu	**4**	**14**	**2**	**Achyk Suu**	**5**	**14**	**2**
Archa Bulak	1	18	3	Archa Bulak	0	19	3
Jaylima	**6**	**13**	**2**	Jaylima	2	19	3
Kabyk	**4**	**15**	**2**	**Kabyk**	**4**	**15**	**2**
Kara Kavak	1	20	3	**Kara Kavak**	**2**	**17**	**2**
Kashka Suu	1	18	2	**Kashka Suu**	**5**	**14**	**2**
Kyzyl Eshme	**3**	**16**	**2**	**Kyzyl Eshme**	**7**	**12**	**2**
Sary Mogul	0	19	2	**Sary Mogul**	**4**	**15**	**2**
Sary Tash	1	18	2	Sary Tash	0	19	2
Taldu Suu	**2**	**17**	**2**	**Taldu Suu**	**4**	**15**	**2**

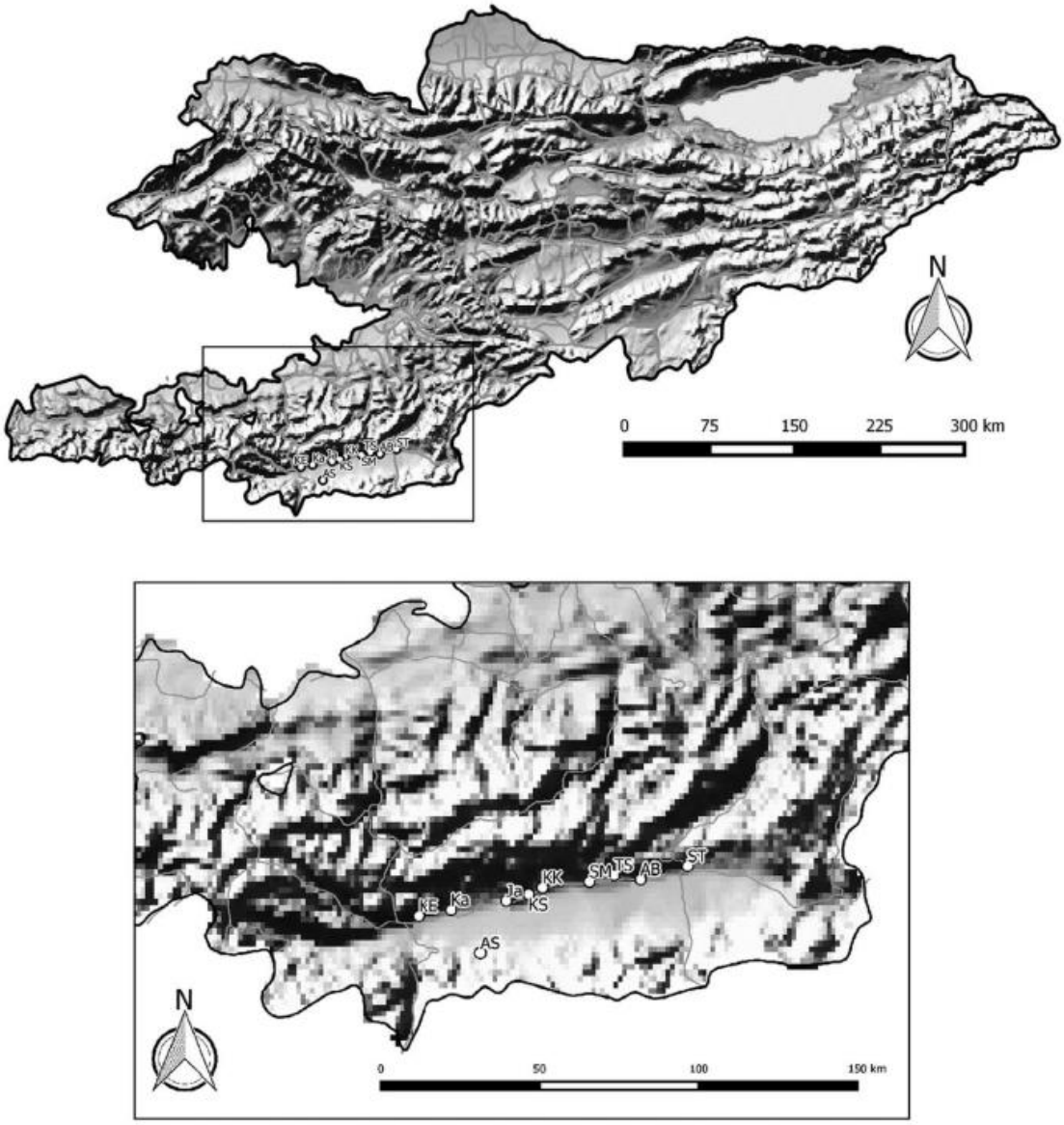
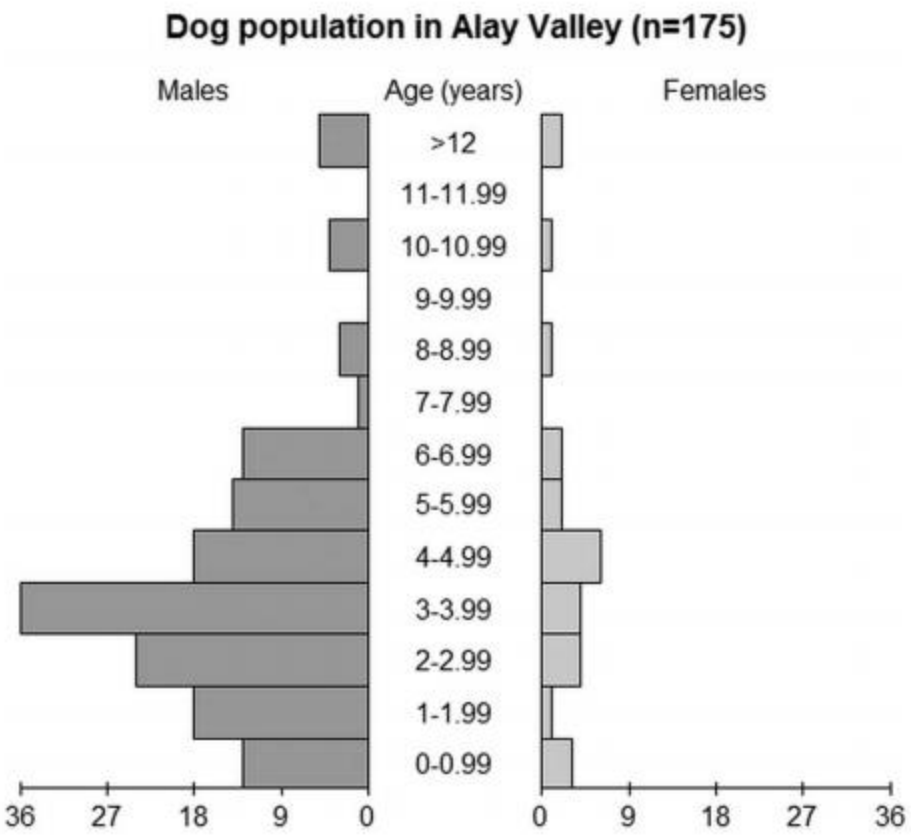
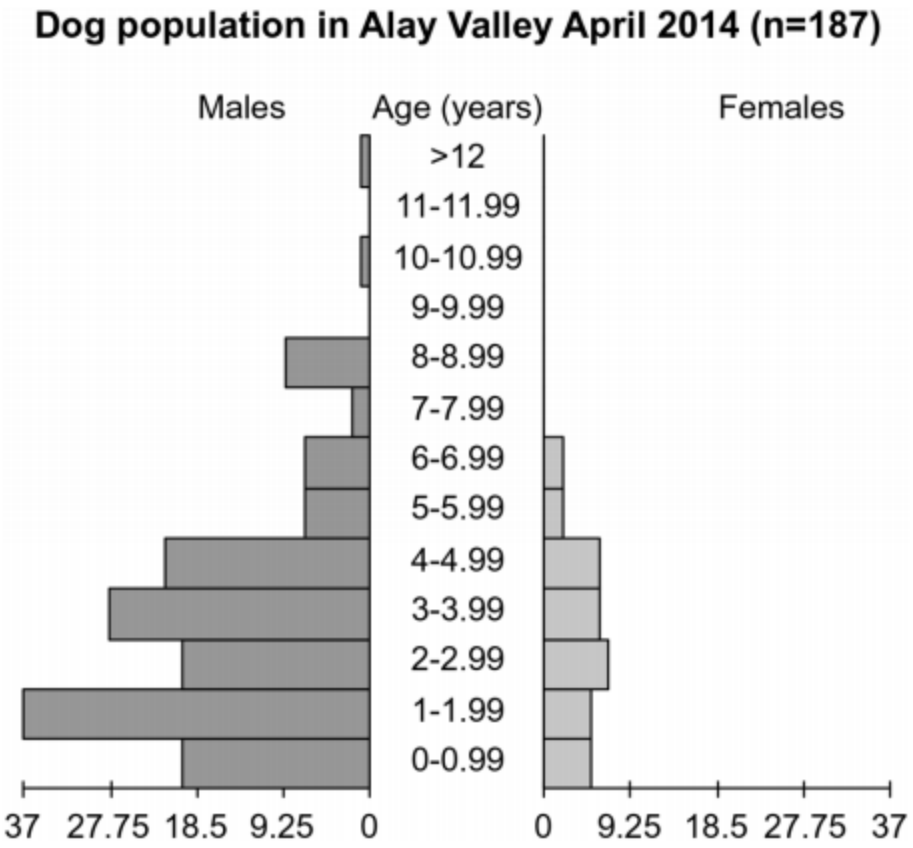


Figure 1: Locations of the study sites within Kyrgyzstan (top) and a region of southern Kyrgyzstan (bottom). KE = Kyzyl Eshme; Ka = Kabyk; AS = Achyk Suul; Ja = Jaylima; KS = Kashka Suu; KK = Kara Kavak; SM = Sary Mogul; TS = Taldu Suu; AB = Archa Bulak; ST = Sary Tash



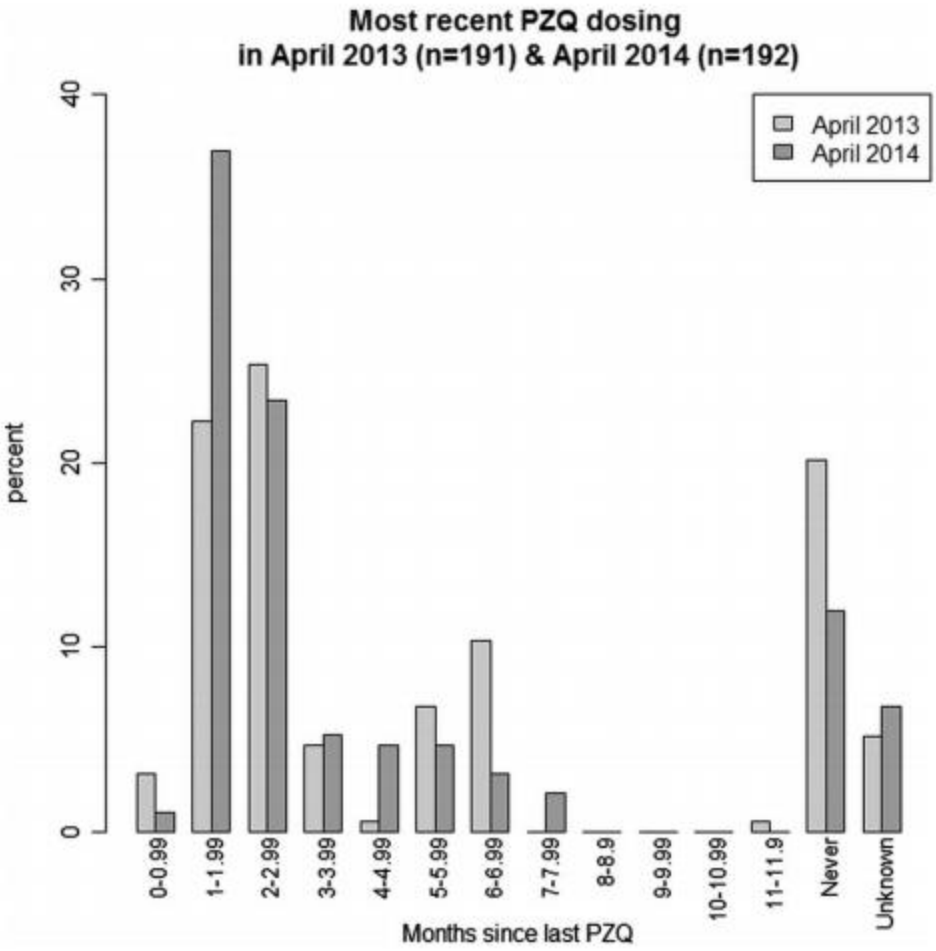
571

572 *Figure 2: Dog demographics in the Alay Valley in April 2013, based on LQAS sampling of ten*
573 *communities. (Note: age and/or sex of 16 dogs not recorded)*



574

575 *Figure 3: Dog demographics in the Alay Valley in April 2014, based on LQAS sampling of ten*
576 *communities. (Note: age and/or sex of 5 dogs not recorded)*



577

578 *Figure 4: Most recent praziquantel dosing for dogs in the Alay Valley in April 2013 and April*
579 *2014*

580